THE OCCURRENCE OF TUMORS ON CERTAIN NICOTIANA HYBRIDS

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With plate 90 and one text figure

INTRODUCTION

TUMORS resembling, in some measure, the outgrowths found in plants infected with the crown gall organism are produced in great abundance by certain *Nicotiana* species hybrids. These curious outgrowths were apparently first described by Kostoff (1930), who carried out a series of experiments which led him to interpret the occurrence of the tumors as a manifestation of immunological activity.

Sufficient data have been accumulated to justify a re-analysis of the situation in respect to these spontaneous tumors and to suggest a plausible explanation for their occurrence on the basis of additional evidence.

The observations reported in this paper have been confined to F_1 plants secured by crossing *Nicotiana glauca* × *N. Langsdorffii*. Without exception plants of this genetical composition produced numerous tumors in some stage of their development.

The F_1 plants of this mating were approximately intermediate in appearance between the parental species. On the whole it may be said that there was little variation among the individuals of the F_1. Chromosome counts secured from root-tip preparations indicated that the chromosome number of these plants was 21. This is the number to be expected considering that the haploid numbers of *Nicotiana glauca* and *N. Langsdorffii* are twelve and nine, respectively. The reduction division in the pollen mother cells of these plants was extremely irregular. Pairing followed the Drosera scheme. Lagging, non-disjunction and other irregularities were frequently observed. The percentage of pollen sterility was very high (96%). Numerous attempts to self-fertilize the F_1 plants resulted in complete failure. Back-crosses to both parental species, utilizing the F_1 as either male or female, have also given negative results. It was not surprising that selfing was unsuccessful in view of the high pollen sterility. Evidently some of the ovules were viable, as Kostoff (1930) obtained back-crosses to *N. Langsdorffii* by employing the F_1 as the female parent.

LOCATION OF THE TUMORS

The tumors emerge on the stem from the axillary leaf bud. Occasionally, they may emerge from the entire leaf scar area. A careful examination of a number of plants has shown that the tumors rarely, if ever, emerge from the internodal spaces of the stem. Repeated attempts to induce tumor formation by scratching and wounding the surface of the stem at locations between leaf scars have thus far failed to induce tumor formation. It has been noted on several occasions that pinching back on the terminal bud seems to stimulate tumor formation by forcing the growth into the lateral, axillary meristematic regions.

Tumors appear to be produced with equal facility on roots or stem. However, in the plants examined, the tumors were more numerous on the stems than on the roots of the plants. In this connection, it is interesting to note that on the stems of the plants under observation, the tumors were emerging from each of the axillary buds from which the leaf had fallen off.

EXTERNAL AND HISTOLOGICAL FEATURES

In external appearance the spontaneous tumors are quite similar to the malformations produced by plants infected with the crown gall organism (Plate 90). When growth is formed into the tumors by pinching or cutting back of the main stem of the plant, a number of young shoots arise from the tumors producing the typical "witches' broom" appearance. It has been observed that the shoots produced in this fashion were identical with the mother plant (judged by external characteristics).

The more or less disorganized tissue making up the greater part of the bulk of the tumors is constituted primarily of parenchymatous cells, associated with scattered vascular elements. As the surface of the tumor is approached one finds numerous small, active meristematic areas. There is a rather thick covering of epidermal hairs over the surface of the tumorous regions.

In common with other malformations of a similar nature, the tumors found on the F_1 plants were characterized by an abundant accumulation of starch and tannin. The starch accumulation was chiefly localized in deposits of large oval masses, occupying an entire cell or in some cases two or three adjacent cells. There is also a considerable quantity of starch grains scattered throughout the entire mass of tumorous tissue. The quantity of starch and plastids in tumorous tissue is very much greater than in normal stem tissue. In comparing the histological features of tumorous tissue with normal stem tissue

one is primarily impressed by the lack of regularity of the former tissue. The scattered vascular elements, isolated starch storage cells and large meristematic surface all serve to heighten the impression of irregularity of the tumor tissue.

PHYSIOLOGICAL CHARACTERISTICS

The evidence on some of the more important physiological characteristics of these tumors can be summed up rather briefly:

1. Inoculation experiments have failed to transmit the causal factor or factors to other organisms. Various species of *Nicotiana* were used in attempt to transmit the causal factor from organism to organism (Kostoff, 1930).

2. It was found to be impossible to isolate bacteria or other parasites from the tumors, although several media were used in culturing attempts (Cleveland, from Kostoff, 1930). It should be pointed out that negative results from inoculation experiments and culturing attempts are merely indicative that parasitic organisms were not present but are not positive proof of this condition.

3. The causal factor cannot be transmitted across the graft union. Heteroplastic grafting, using the F_1 in combination with both parent species, utilized as both stock and scion, have failed to transmit the causal factor across the graft union. In combination with other species of *Nicotiana* (*N. Tabacum, N. paniculata*), the F_1 (*N. glauca* × *N. Langsdorffii*) has not been able to transmit the tumor-inducing agent across the graft union. Thus it seems abundantly proved that the tumors cannot be induced in either of the parent species, or in the two other species of *Nicotiana* used in the experiments, by means of grafting.

4. In the plants under observation the tumors developed on the roots during the early seedling stage. Tumors usually begin to appear on the stem during the flowering period, and once tumor formation is initiated, it continues throughout the remainder of the plant's life. In one family of F_1 plants there were five individuals which appeared to have symptoms of tumor on the stem at a very early age. These plants did not develop past the rosette stage and made no attempt to send up a flowering shoot but continued to produce sessile leaves in a crowded fashion, upon a much reduced stem. Root-tip counts of the chromosomes of these plants showed them to be 21 in number, similar to sister plants which were not deformed.

In connection with time-development, it is well to note that as long as the plant is in active growing condition and the terminal meri-

stematic region is active, tumors do not occur on the stem. When the plant has reached a certain physiological age and meristematic activity in the tip region has ceased, this activity is transferred to the lateral axillary buds, and tumor formation begins. The time when the plant begins to flower usually coincides with the time at which terminal meristematic activity ceases and tumor formation is initiated.

5. The tumors are apparently not malignant, at least not in the ordinary sense. Plants having tumors have been kept in good condition for over a year without any particular attention. These plants have never flowered after the initiation of tumor formation but the vegetative growth does not seem to have been impaired.

KARYOLOGICAL OBSERVATIONS

The chromosome number of the cells making up the tumorous areas of the plant can be very easily and quickly determined by the aceto-carmine smear maceration method. The technique used is the same as that employed in making root-tip smears (Whitaker, 1934), except that in making smears of tumor tissue, it is preferable to bring the material to boil in aceto-carmine before smearing. The latter treatment is a distinct aid in obtaining satisfactory maceration of the tissue.

FIGURE 1. Microphotograph of an aceto-carmine smear of tumor tissue (× 2000). The number of cells in various stages of mitosis indicate the active state of reproduction often found among the cells of the meristematic areas.

The chromosome number situation is interesting in view of the findings of Winge (1927) in crown galls produced by *Bacterium tumefaciens* on the sugar beet. Winge has made observations which seem

to indicate that the majority of the tumorous cells are polyploid. He has interpreted this evidence as supporting the hypothesis that tumors result from somatic polyploidy.

The chromosomes in over 40 cells of the tumorous tissue have been counted, and not a single polyploid cell was found. Occasional tetraploid cells have been encountered in other slides but these are undoubtedly very rare.

Mitosis in cells of the tumor tissue seemed to be very regular; there was seldom any pronounced lagging or other irregularity (Text fig. 1). The few irregularities that have been found have not been considered significant.

In examining cells making up the tumor, particular attention was devoted to the nucleus in the hope of detecting karyological abnormalities which might possibly give some clue to the solution of the problem. The percentage of polynucleated cells is almost negligible. Three or four cases of double nucleated cells were recorded, but in only one of these cases could it be positively demonstrated that two nuclei were present in the same cell.

The status of the nucleolus in the tumorous tissue does not vary significantly from that of normal stem tissue. In the normal stem tissue, nuclei with as high as four nucleoli have been observed. Variations within this number have also been found in tumor tissue. The majority of the nuclei in tumor tissue seem to have two nucleoli.

DISCUSSION

In view of these observations, three possible causes of tumor formation have been considered.

1. IS THE CAUSAL FACTOR A VIRUS, BACTERIUM OR OTHER MICROORGANISM? This question is most effectively answered by the grafting experiments. It will be recalled that in these experiments, efforts to transmit the causal factor by grafting were unsuccessful. Many of the known viruses are readily transmissible across the graft union. (Heald, 1926). Further support against the supposition that the causal factor may be a virus is evident from the appearance of the plants which do not exhibit any of the typical known virus symptoms.

The inoculation experiments seem to rule out the possibility of the causal factor being in the nature of a bacterial or fungal parasite. Microscopic observation of both fresh and fixed material points in the same direction; namely, that bacterial and other parasites cannot be regarded as the responsible agents in tumor formation.

2. THE IMMUNOLOGICAL INTERPRETATION. This solution of the cause of spontaneous tumor formation in *Nicotiana* species hybrids has

been advocated by Kostoff (1930). His critical evidence in favor of this view is the "precipitin reaction" occurring between the parent species. The immunological significance of this reaction has been discussed (Chester and Whitaker, 1933). It is sufficient to state that this is not a critical test.

This is a difficult hypothesis either to prove or disprove. The only fact bearing on this question, and it is in no sense critical, is that *N. Langsdorffii* can be used as both stock and scion in graft combinations with *N. glauca*. They appear to be a fairly compatible graft combination. One would think that if species specific substances were diffused by each graft partner, the combination would be an extremely temporary affair. Such is not the case. These combinations are as long-lived as comparable, known compatible combinations and show no ill-effects from their intimate association.

Although the immunological interpretation cannot be eliminated by any of the known facts at the present time, it is a little difficult to visualize a system by which it would work in this instance. It might be possible that if it were an antigen-antibody reaction, the effect would be to limit the hybrid embryo to a few cell divisions, as McCray (1933) has found to be the case in some *Nicotiana* hybrids.

3. A CYTOPLASMIC DISTURBANCE CAUSED BY THE INTRODUCTION OF THE MALE CHROMOSOME COMPLEMENT. In this interpretation of the cause of tumor formation, the assumption is that the disturbance produced by the nine chromosomes of *N. Langsdorffii* in the cytoplasm of *N. glauca* is directly responsible for uncontrolled growth in certain tissues of the plants. This uncontrolled growth is exhibited externally by the tumors. An examination of the evidence indicates that this theory has some merit.

The first essential to have in mind is that the tumors are produced only when *N. glauca* is used as the female parent and *N. Langsdorffii* as the male parent. The reciprocal cross, although difficult to make, has been obtained by McCray (1932). The plants were very vigorous, and there was considerable variation among them. Even though there was a marked amount of variation among the F_1 individuals there was no reason for thinking that this was not a legitimate F_1 between the two species. From this work it seems to be clear that a reciprocal F_1 has been produced (*N. Langsdorffii* used as the female), and tumors were not present (McCray, private correspondence).

Theoretically if a back-cross to either species could be obtained, it should yield critical data on the validity of the proposed explanation. Back-crosses to the *N. Langsdorffii* parent have been made by employ-

ing the F_1 as the female parent (Kostoff, 1930). The progeny of such a mating should all possess tumors in a greater or less degree, since in such a system of matings no *N. Langsdorffii* cytoplasm has been introduced. It is not clear from Kostoff's description of the progeny of this mating whether all the plants had some indication of the disturbance. Apparently the majority of them had fasciations, which appear to be a milder expression of the disturbance than the spontaneous tumors. A smaller number of these plants had tumors, reported to be as large and as numerous as the tumors formed on the F_1.

The time of development does not offer serious difficulties in the path of the above explanation. The tumors are present in the very early seedling stage, at least on the roots. They were first visible on the stem about the time of flowering. If the effect is that of chromosomes upon cytoplasm, one would rather expect that it would exert a continuous influence from the earliest divisions following fertilization on through the life of the individual. The first indication of spontaneous tumors occurs in the roots where they are found shortly after germination. This seems to indicate that there is a continuous expression of the disturbance from immediately after germination on through the remainder of the life of the individual.

We have examined the evidence bearing on the explanation of tumor formation in the F_1 of *Nicotiana glauca* × *N. Langsdorffii*. How does this fit in with other cases of tumor occurrence reported in hybrid Nicotianas?

The cases in which tumors have been reported are listed in Table I.

TABLE I

Chromosome No. (n)			Chromosome No. (n)	Investigator
N. quadrivalvis	24	× *N. Sanderae*	9	Holmes (private correspondence)
N. rustica	24	× *N. Sanderae*	9	Kostoff
N. Tabacum wigandioides	24	× *N. Sanderae*	9	Kostoff
N. paniculata	12	× *N. Sanderae*[1]	9	Kostoff
N. paniculata	12	× *N. Langsdorffii*	9	Kostoff
N. rustica	24	× *N. Langsdorffii*	9	Kostoff
N. rustica	24	× *N. alata*	9	Kostoff
N. Tabacum sanguinea	24	× *N. alata*	9	Kostoff

[1] *N. paniculata* × *N. Sanderae* must be considered as a doubtful case for the reason that the tumors did not appear on the stems, and it was questionable whether there were tumors present similar in nature to the cases cited above. Moreover, Christoff (1928) has produced this same F_1 combination, and in describing the plants, he makes no mention of the occurrence of tumors.

It is evident, from an inspection of Table I, that, in the combinations producing tumors in the F_1, the alata-group (*Nicotiana alata*, *N. Sanderae*, and *N. Langsdorffii*) having nine chromosomes is always involved as the male parent. There is one possible exception that should be mentioned. Kostoff (1930) records the F_1 hybrid of *N. rustica humilis* × *N. Tabacum sanguinea* as producing malformations, causing it to be placed with the tumor-producing hybrids of *Nicotiana*. It is distinctly questionable whether it belongs in this category. Tumors were not present on the stem or if present were not noticeable. A slight fasciation was produced which may or may not have been similar to the fasciations produced by the truly tumorous F_1 hybrids. The argument advanced above may well serve to account for tumor formation until more information on the progeny of this cross is available.

The explanation of tumor formation that has been offered, in which the cause has been assumed to be the disturbance originating from the introduction of *N. Langsdorffii* chromosomes into *N. glauca* cytoplasm, can be very easily extended to include the alata-group. The evidence presented in Table I signifies that the same forces are at work in this group of F_1 species hybrids as were found in the F_1 progeny of *N. glauca* × *N. Langsdorffii*.

In cases where reciprocal crosses have been obtained (alata-group used as the female parent) tumors have not been recorded as being present on any of the plants. For the reason that it is somewhat easier to secure crosses in which the species with the higher chromosome number is used as the female parent, most of the reciprocal crosses have not been successful, but where it has been possible to secure fertile crosses and hybrids brought to maturity, tumors were not reported in the description of the F_1 plants. The two cases where reciprocals have been obtained are: *Nicotiana Langsdorffii* × *N. Tabacum* and *N. Sanderae* × *N. Bigelovii* (from East, 1928). The fact that tumors were not recorded by the observers cannot be taken as absolute proof that they were not present, but there is the greatest likelihood that if tumors were present they would have been at least mentioned. It, therefore, seems safe to assume that these reciprocal F_1 hybrids were devoid of tumors.

One major criticism that can be made of the cytoplasmic disturbance theory, when applied to the three species belonging to the alata-group, is that the reactions of all three species are not the same when mated to a common species as far as the production of tumors is concerned. As an example of this point, take the cases where *Nicotiana glauca*

has been used as a female with *N. alata*, *N. Sanderae*, and *N. Langsdorffii*. In the first two cases the F_1 does not produce tumors. In the last case the F_1 produces tumors in great abundance. If we were dealing with a comparatively simple phenomenon, it seems reasonable to expect that all of the alata-group, when mated to a common species (*N. glauca*), would have similar reactions. That such is not the case has been pointed out above.

The evidence is fairly conclusive that when the 9-chromosome group of *Nicotiana* is introduced by the pollen parent into the cytoplasm of certain other *Nicotiana* species, there occurs a loss of control of growth by the organism in the meristematic areas. As a result of uncontrolled growth, tumors appear first on the roots and later on the axillary buds of the stem.

SUMMARY

1. Spontaneous tumors occurring on F_1 species hybrids between *Nicotiana glauca* and *N. Langsdorffii* have been studied from several different viewpoints in an attempt to discover the underlying causes for their occurrence.

2. All the available evidence indicates that the tumors are the direct result of a cytoplasmic disturbance. The disturbance is occasioned by the introduction of the chromosome complement of *N. Langsdorffii* (used as the pollen parent) into the cytoplasm of *N. glauca* (used as the seed parent).

3. The reported cases of spontaneous tumors in the F_1 of species hybrids of *Nicotiana* have been analyzed. All of them seem to be due to a cytoplasmic disturbance caused by the introduction of the chromosomes of the alata-group (*N. alata*, *N. Sanderae*, and *N. Langsdorffii*) used as the male parent into the cytoplasm of certain other Nicotianas (*N. glauca*, *N. paniculata*, etc.) used as the female parent.

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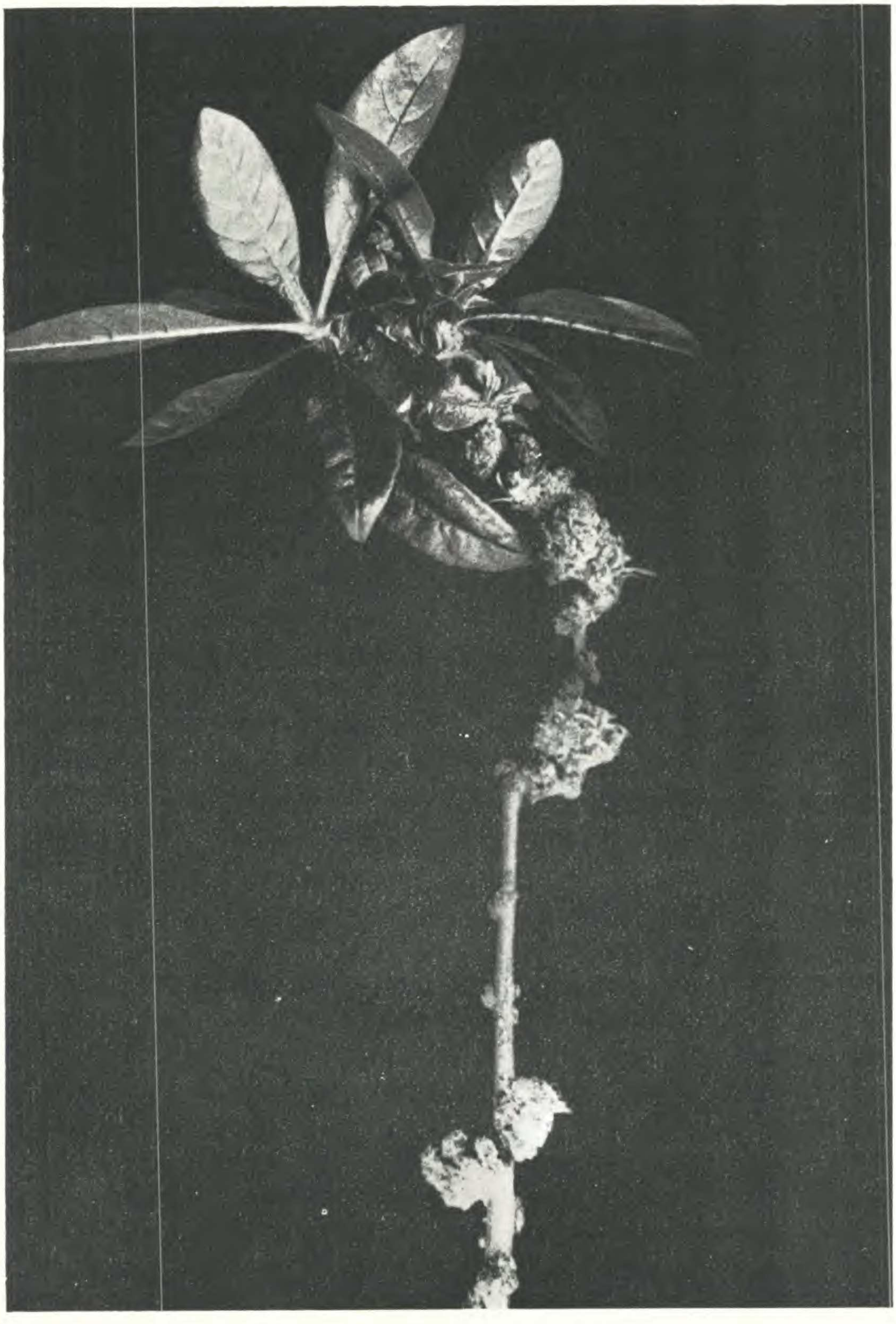

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